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Determining Fetal Pulmonary Maturity by Testing Surfactant in Amniotic Fluid

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The chances of a newborn infant developing respiratory distress syndrome (RDS) rests in part on the formation of surfactant in his lungs. Since this substance escapes to the fetal sac, its concnetration in the fluid forms an efficacious tool for measuring pulmonary maturity prior to birth.

A simple, rapid, and inexpensive test was first devised by Clement (2) to determine the presence of surfactant material in the amniotic fluid. The test depends on the ability of the surfactant to generate stable foam in the presence of ethanol.

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The reliability of this test is proven by the fact that results become more positive with the advancement of gestation. The result was found to be negative in 100% of the cases which were prior to 28 weeks' gestation, positive in 66.8% of the cases in pregnancies of 29-34 weeks' gestation, positive in 89% of 30-4- weeks', and positive in all cases beyond 40 weeks' gestation.

In 93 cases the test was administered in order to predict before birth the possibility of the appearance of RDS in the infant. It was found that in samples with clearly positive results (a large, positive calibration, and equal to 1/1.3), not one infant developed RDS. On the other hand, in cases with clearly negative tests, all 3 infants developed RDS, and 2 even died from the disease.

In cases with intermediate tests (a measurement of only 1/1), it was preferable to wait until the measurement rose. The test is also utilizable and an aid in diagnosing post-term preganancy since in all cases where the pregnancy was over the 41st week, the result were clearly positive.

The principal reason for the infant mortality rate in the first days of their life is prematurity. In a majority of cases, the reason for death is the respiratory distress syndrome (RDS) that appears in approximately 17% of all births weighing less than 2,500 grams. 20-30% of all infants in which RDS appears die (1). These facts explain the efforts of researchers to discover means for preventing the disease and ways to cure it.

The reason for RDS is still unknown, but it appears that in these cases the alveolar surface tension remains high after exhalation, causing the collapse of the vesicles as well as increased shrinkage.

Avery and his colleagues (3), in their examination of specimens of premature lungs that suffered from RDS, found that they lacked the ability to reduce this tension. It was found that the mature ones had such a capacity. The spread of the lung vesicula prevents a telactasis. The researchers raised the conjecture that the disease results from lack of, or lateness in appearance of, the functional pulmonary system. They have even assumed that the synovia from the disease can appear when the maturation of the outer cells (vesicula) cease in the first days following birth. The exact chemical composition and concentration of components active in the lungs is not known, but it seems that the basic component is Dipalmityl phosphatidil choline (DPC), known commonly as lecithin. This lipid reduces the internal tension that is most frequent in the outer cells of the vesicles of the lung. DPC is synthesized in the lungs and is not present in any other part of the body in any discernable quantity; it appears in increasing concentration in every infant as gestation progrsses. In infants that died from RDS, the concentration of lecithin was signficantly lower than in infants that died from other diseases (5, 6). Since a drift was found from the fetal tracheobronchial tree to the amniotic fluid, it could be assumed that the concentration of lecithin in amniotic fluid would reflect the degree of maturity of the fetal lungs.

Gluck and his associates (7) proved in 302 cases of amniocentesis that change in concentration of the phopholipids (determined by using the lecithin-sphingomyelin ratio) in amniotic fluid as the gestation progresses, reflects the maturation of fetal lungs.

Because of the aforementioned discoveries, interest has increased in the ability to predict from the beginning the possibility of the infant developing RDS, according the relative concentrations of lecithin and sphingomyelin in the amniotic fluid, and the results are encouraging (1). Recently a rapid, simple, and inexpensive method has been developed to determine the presence of surface-active material in the amniotic fluid, by the ability of the fluid to generate stable foam in the presence of ethanol. This test (using the lecithin-sphingomyelin ratio) for predicting the possibility of the fetus' developing RDS after birth has been found to be effective.

In view of the above work, if gestation is advanced and the presence of surfactant in the amniotic fluid is proven, the test is reliable for predicting the appearance of RDS.

PROCEDURE

In five clean and dry tubes with diameters from 1.0 to 1.5 cm. and labeled 1/1. 1/1.3, 1/2, 1/4, and 1/5, there is pippited amniotic fluid in volumes of 0.20, 0.25, 0.50, 0.75, and 1.0 ml. Saline solution in volumes of 0.25, 0.50, 0.75 and 0.80 were added to four tubes to bring the total volume to 1 ml. in each tube, and i milliliter of 95 per cent ethanol is also added to each of the 5 tubes.

The tubes are capped with clean rubber stoppers and shaken vigorously of 15 seconds and then stood upright in a rack. After 15 minutes they are examined for positive results, whether there is now found small, stable bubbles that are like those in nature on a brook's surface. Analysis is made against a black background. Interpretation of the test

bubbles can follow one the these possibilities:

A) A clearly positive test is when a complete ring of stable foam is seen, measuring 1/1/3 and more. This result is thought to indicate the maturity of fetal lungs and allows for inducing labor in cases which indicate it.

B) A clearly negative result is when no ring of stable foam appears, not even one measuring 1.1. This result indicates the immaturity of the lungs and the great possibility of developing RDS.

C) An intermediate result is when the resulting ring only measures 1.1.

The necessary technic for the success of the test:

A) Ensuring the cleanliness and dryeness of the instruments

B) Precise concentration of ehtanol

C) Not moving the tubes after the foam is produced, since the bubbles could break.

D) The test is not effective when meconium, blood, talc or other substances are present.

E) Centrifugation of the fluid at high speed or for long periods should not be done, since this might cause the lipids to settle.

EVALUATION AND RESULTS

The trial was divided into 2 levels. In the first, the changes in the concentration of the surfactant in amniotic fluid (according to examination of the foam) at different weeks of gestation were examined. The age of the pregnanacy was determined for the most part according to the time of the last menstrual period, and in a small number of cases

according to the day of conception.

In 37 women on whom amniocentesis was performed according to the accepted method (8), part of the amniotic fluid was taken for immediate examination of the foam. 16 women were at 28 or less weeks' gestation, 12 were 28-34 weeks', and 9 were at up to 36 weeks. In 33 cases the amniotic fluid was obtained at a time when the uterus was pierced as part of a paracervical bloc to induce labor in different pregnancies.

The women were divided into 4 groups according to the stage of gestation:

1. Gestation stage of 28 weeks or less
2. Gestation stage of 29-34 weeks
3. Gestation stage of 35-40 weeks
4. Gestation stage of 41 weeks or more (post-term pregnancies).

The results are shown in Fig. 1.

The negative results were found in all pregnancies under 28 weeks. The percentage of positive results rises progressively with advancement of gestation and reaches 66.8% in pregnancies of 29-34 weeks, 89% in those of 30-40 weeks, and 100% in those over 40 weeks. In the second level of the experiment [illegible].

The test underwent clinical examination. The order is explained in Table 1.

In 90 pregnancies, amniotic fluid was obtained by trans-abdominal puncture of the uterus. In the 3 cases of premature birth, the fluid was obtained transvaginally as part of a paracervical bloc.

Table 2 illustrates the cases according to the results of the testing for stable foam.

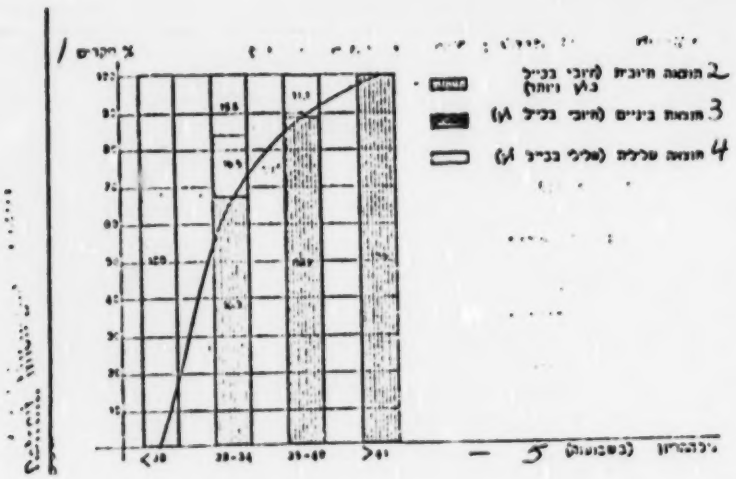

Fig. 1. Percentage of positive results in test for stable foam, with cases broken down according to age of gestation.

Key:
1. % of cases
2. Positive result (positive in the amount of 1/1.3 and more)
3. Intermediate results (positive in the amount of 1/1)
4. Negative result (negative in the amount of 1/1)
5. Stage of gestation

Table 1. Order of testing for stable foam

Result	Number of cases
a) Complete maturity (post-term pregnancy)	32
b) Ceasarian delivery	11
c) Recurrent Ceasarian delivery	8
d) Labor induced because of:	
1. Toxemia	17
2. Known diabetes	2
3. Pregnancy-connected diabetes	8
4. RH-incompatibility	3

e.	Premature breaking of waters	5
f.	Bleeding in the third trimester	3
g.	Premature birth	3
h.	Other	1
	Total	93

A) A positive test measuring 1.13 and more was found in 57 cases. This result is thought to indicate the maturity of the fetal lungs and therefore labor was induced in every case. The average weight of the infants was 2,640 grams and none developed RDS.

B) Negative test - The absence of foam in all the tests was found in 4 women. 3 of them, of course, were in an active stage of labor. The average gestation was 28 weeks, and in all 3 childbirth was spontaneous and within 6 hours of the test. The average weight of the infants was 1,160 grams and all developed RDS.

Two died from the disease. The fourth, 39 weeks of gestation at birth, was a case of hydromeninx: a male born weighing 3,140 grams, with severe meningomyelitis. The infant did not develop RDS, but died from the birth defect.

C) Intermediate tests (positive test at a measurement of only 1/1) were found in 8 cases. In 7 of them we waited from 1 to 3 weeks for a positive test in the examination of the foam. In these 7 cases RDS did not appear. In the eighth case, in spite of the inconclusive results, labor had to be forced in the 35th week

of gestation because of a radical outbreak of previously undetected diabetes in the mother. A male was born weighing 2,040 grams and died after 3 days from RDS.

Table 2: Condition of newborn (appearance of RDS) compared to results from testing for stable foam.

1/ מיבחן יציבות הבועות		1c מס' המקרים	1d גיל הריון ממוצע	1e משקל ילוד ממוצע	1f תופעה קיומי ת"י	1g תמותת ילדים
1a תוצאה	1b פירוש					
2 א. חיובית בכייל של 1/1.3 ויותר	3 בשילית ריאתית	5	35	2.540	—	—
4 ב. חיובית בכייל של 1/1 בלבד	5 ההשאת־ביניים	8	38	2.890	1	—
6 ג. שלילית בכל המבחנות	7 חוסר בשילית ריאתית	3	39	1.160 (3.140)	3	2 (+1)
8 ד. חיובית בכייל של 1/5	9 הריון עודף	32	42	3.730	--	—

Key:
1. Test for stable foam
1a. Result
1b. Explanation
1c. Number of cases
1d. Average gestation age (at birth, in months)
1e. Average weight of newborn
1f. Appearance of RDS symptoms
1g. Infant deaths
2. Positive in the amount of 1/1.3 or more
3. Maure lungs
4. Positive only in the amount of 1/1
5. Intermediate
6. Negative in every test
7. Immature lungs
8. Positive in the amount of 1/5
9. Post-term pregnancy

D) In 24 out of 32 cases of post-term pregnancies, which were 41-43 weeks, a positive result was found in all 5 tests. The average weight of the infants was 3,730 grams and in some of them indications of post-term pregnancy were found. None of them developed RDS.

DISCUSSION

Determining fetal maturity is important in obstetrics in general and in different diseases of the pregnant woman and especially the pregnancy in which there are indications of premature delivery. Among these diseases especially are diabetes, toxemic pregnancy, cases of the fetal sac bursting, bleeding the third trimester of pregnancy, discharge from the placenta, high blood pressure, kidney diseases, and cases of RH-incompatibility. All of these diseases can interfere with the growth and developement of the fetus. The aim of induced deliveries is to prevent irrreversible damage to the fetus or its death during the remainder of its time in the womb. On the other hand, premature delivery prior to maturity can expose the infant to RDS, resulting in no less danger than had it remained in the womb. RDS in addition to immaturity can be the surprising finding in deliveries that had been anticipated to be post-term and also in therapeutic and spontaneous abortions in which the extent of gestation is unclear.

From everything stated above, it is understandable why such tremendous effort has been invested in the extensive research to find methods for measuring fetal maturity before delivery. Among the tests that have been developed: determining the time of the appearance of distal femoral epiphysis (9); measuring the fetal biparietal diameter by use of sonargrams (10); checking concentrations of amniotic fluid by the concentration of cells dyed Nile Blue (8); and determining levels of bilirubin and creatinine (8). All of these tests perhaps give some indication of the size of the fetus; however they do not allow the measurement of the maturity of the fetal lungs, which is what determines the appearances of

of RDS. A big step was made by Gluck and his associates (7), that demonstrates the relationship between the concentration of lecithin (the material which reduces alveolar surface tension) in the amniotic fluid and the maturity of the lungs. Testing the lecithin-sphingomyelin ratio is generally complex and not practicable in every obstetrics department. Testing for stable foam also depends on the presence of surface-active material in the amniotic fluid but it is a rapid test, inexpensive and much simpler than determining the lecithin-sphingomyelin ratio.

The reliability of the test for stable foam in determining the presence of surfactant in the amniotic fluid as well as fetal pulmonary maturity was demonstrated in experiment No. 1 (Fig. 1). During the weeks in which all fetuses lack pulmonary maturity (less than 27 weeks' gestation), the test showed negative results in 100% of the cases.

The percentage of positive results steadily rises and reaches 66.8% at gestations of 29-34 weeks, 89% in gestations of 35-40 weeks, and 100% beyond the 40th week.

After proving its reliability, the method was used in cases involving situations where premature delivery was indicated, and in 3 of spontaneous premature delivery (Table 1). The effectiveness of testing for stable foam in predicting the appearance of RDS was demonstrated by the situation of the infants in these cases. Classified into 4 groups according to the results of the foam tests, it was found that in the first group, in which the results were positive (measuring 1/1.3 or more, indicating pulmonary maturity), RDS did not appear at all, nor was there infant mortality.

The second group are the negative results in all tests, evidence of fetal pulmonary immaturity. There were 4 cases of RDS. 3 of them were

premature births; 3 infants developed severe symptoms of RDS and 2 died from the disease. In the fourth case, in which there was an extreme abundance of amniotic fluid (12 liter), the infant weighed 3,140 grams and suffered from severe meningomyelitis that could not be cured. It died after a few days, with no signs of RDS. There is no obvious explanation of the negative results in spite of pulmonary maturity, but 2 possible explanations are:

1) Adulteration of the surfactant in the large volume of amniotic fluid
2) Because of the crucial defect, it is possible that it interfered with the movement of the fluid from the fetal tracheobronchial tree, in contrast to the normal method of liquid movement.

In the third group, intermediate results measuring only 1/1 positive, there were 8 cases. In 7 of them the inducing of labor was postponed for an additional period, and in the eighth case there was no possibility of postponing labor due to the presence of severe complications in the diabetic mother, in the 35th week of gestation. The birth was obscure. A boy was borm weighing 2,040 grams and developed severe symptoms of RDS 6 hours after birth, dying from the disease 3 days later.

Of especial interest is the fourth group, post-term pregnancies, in which the test was always positive. Certainly such a positive result can be used as an effective tool in diagnosing post-term pregnancies.

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